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Mdr1b facilitates p53-mediated cell death and p53 is required for *Mdr1b* upregulation *in vivo*

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The mdr1b gene is thought to be a "stress-responsive" gene, however it is unknown if this gene is regulated by p53 in the whole animal. Moreover, it is unknown if overexpression of mdr1b affects cell survival. The dependence of mdr1b upon p53 for upregulation was evaluated in p53 knockout mice. Wild-type (wt) or p53−/− mice were treated singly or in combination with gamma irradiation (IR) and/or the potent DNA damaging agent, diethylnitrosoamine (DEN). Both IR and DEN induced *mdr1b* in wild-type animals, but not in the p53−/− mice. IR also upregulated endogenous *mdr1b* in the H35 liver cell line, and the *mdr1b* promoter was activated by IR and activation correlated with p53 levels; moreover activation required an intact p53 binding site. Colony survival studies revealed that co-transfection of both mdr1b and p53 dramatically reduced colony numbers compared to cells transfected with either p53 or mdr1b alone and cells microinjected with both mdr1b and p53 had a more dramatic loss in viability compared to cells injected with either expression vector alone. Further studies using acridine orange and ethidium bromide to measure apoptosis revealed that mdr1b caused apoptosis and this was enhanced by p53, however the increased apoptosis required a functional p53 transactivation domain. These studies indicate that mdr1b is a downstream target of p53 in the whole animal and expression of mdr1b facilitates p53-mediated cell death. *Oncogene* (2001) **20**, 303–313.

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Introduction

Mammals possess plasma membrane ATP dependent transporters that can confer resistance to structurally diverse amphipathic cytotoxic agents (Gottesman and Pastan, 1993). While the human *MDR1* gene represents the prototype member, genes that are highly related have been cloned from rodents (Gottesman and Pastan, 1993). The rodent *mdr1a* and *mdr1b* genes share greater than 88% identity, are often expressed in the same tissues and can be selectively overproduced during selection for resistance to cytotoxic agents (e.g., taxol, vinblastine, adriamycin (Lothstein *et al.*, 1989)). Further, selection for drug resistance with ectopically introduced *mdr1a* or *mdr1b* expression vectors yields similar drug resistance profiles for many drugs (Devault and Gros, 1990). In contrast, the application of cytotoxic drug selective pressure to murine macrophage cells caused a switch in endogenous mdr expression from predominantly *mdr1b* to *mdr1a* (Lothstein *et al.*, 1989; Lin *et al.*, 1995). The basis for the switch is a presumed transcriptional upregulation of *mdr1a*. Although, the mechanism accounting for this conversion is unknown, it may be hypothesized that high levels of mdr1b are deleterious to cell survival, a concept that is consistent with studies showing that cytotoxic drugs that are not mdr1 substrates as well as oxidative stress induce *mdr1b* (Ziemann *et al.*, 1999; Thevenod *et al.*, 2000) and that transcriptional upregulation of mdr1b has been correlated with decreased viability (Schrenk *et al.*, 1996).

Drug sensitivity studies reveal, with respect to drug substrates, almost identical functions between mdr1a and mdr1b (Lothstein *et al.*, 1989). This redundancy in drug transport seems unnecessary, especially considering that both genes are co-expressed in many tissues, and in some cases at similar levels (e.g., liver, adrenal, colon and lung) (Schinkel *et al.*, 1995). These findings suggest that mdr1a and mdr1b have additional biological functions independent of drug transport. Indeed, recent studies revealed functional differences between mdr1a and mdr1b (Valverde *et al.*, 1996). In particular, mdr1a increases the rate of activation of swelling-activated chloride channels in response to hypo-osmotic conditions, thereby facilitating restoration of cell volume after exposure to hypo-osmotic stress (Valverde *et al.*, 1996) (human MDR1 is mdr1a's functional orthologue in this respect). In contrast, it has been reported that cells overexpressing mdr1b have an impaired ability to activate chloride channels in response to hypo-osmotic conditions (Bond *et al.*, 1998). Further studies by several groups have suggested that MDR1 plays a role in apoptosis. For example, ectopic overexpression of MDR1 diminishes the

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